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(54) Title: USE OF LIPOXIN A_4 AND ITS DERIVATIVES AS ANTAGONISTS FOR SLOW-REACTING SUBSTANCES OF ANAPHYLAXIS

(57) Abstract

Composition and methods are disclosed for antagonizing SRS-A, and especially LTD_4, comprising the administration of LXA_4 or an active derivative thereof to an animal. These compositions and methods are useful in the control of hemostasis, vascular reactivity, and especially vasoconstriction, and anaphylactic and allergic reactions in animals.

DESIGNATIONS OF "DE"

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USE OF LIPOXIN A4 AND ITS DERIVATIVES AS ANTAGONISTS FOR SLOW-REACTING SUBSTANCES OF ANAPHYLAXIS

Field of the Invention

This invention is in the area of vasodilators. Specifically, this invention is directed to medicinal compositions comprising lipoxin A4 (LXA4) or derivatives of this compound and their use as leukotriene D4 (LTD4) receptor antagonists to alleviate leukotriene-mediated vascular constriction. These compositions and methods are useful in the treatment of hemostasis, vascular reactivity and inflammation, and especially anaphylactic and allergic reactions in animals.

Background of the Invention

Lipoxygenase products of arachidonic acid (eicosatetraenoic acid) display a diverse array of biological activities (Samuelsson, B., et al., Science 237:1171-1176 (1987)). At least four major classes of lipoxygenase-derived products of arachidonic acid metabolism have been identified: hydroperoxides (for example, 12-HPETE, 15-HPETE), non-peptidic leukotrienes (for example, LTB4), sulfidopeptide leukotrienes (for example LTC4, LTD4, and LTE4) and lipoxins. Generally, these classes are grouped together as lipoxygenase-derived eicosanoids

(LDE); however, each class has a distinct profile of biological activities.

Lipoxins (lipoxygenase interaction products) are a novel series of arachidonic acid-derived metabolites which have been characterized only recently (Serhan, C.N. et al., Biochem. Biophys. Res. Commun. 118:943-949 (1984); Serhan, C.N. et al., Proc. Natl. Acad. Sci. USA 81:5335-5339 (1984)). The distinguishing feature of the lipoxin chemical structure is the presence of a trihydroxy conjugated tetraene structure. At least two biologically active lipoxins have been characterized; LXA$_4$ ((5S,6R,15S)-5,6,15-trihydroxy-7,9,13-trans-11-cis-eicosatetraenoic acid) and LXB$_4$ ((5S,14R,15S)-5,14,15-trihydroxy-6,10,12-trans-8-cis-eicosatetraenoic acid (Serhan, C.N. et al., J. Biol. Chem. 261:16340-16345; Serhan, C.N. et al., Proc. Natl. Acad. Sci. USA 83:1983-1987 (1986)).

Very little is known about the biological roles or activities of the lipoxins. It has been suggested to use LXB$_4$ to treat diseases characterized by inflammation mediated through the activation of neutrophils such as that found in asthma, arthritis, physical trauma and radiation exposure (Morris, J., U.S. 4,576,758; Samuelsson, B. et al., U.S. 4,560,514).

LXA$_4$ has been shown to contract pulmonary smooth muscle (guinea pig lung), but not guinea pig ileum or trachea, and to relax (dilate) vascular smooth muscle at concentrations of less than 1 μM (Dahlen, S.-E. et al., Acta Physiol. Scand. 130:643-647 (1987)). Topical administration of LXA$_4$ to the hamster cheek pouch induces a pronounced arteriolar dilation, but does not change venular diameters (Dahlen, S.-E. et al., in Adv. Exper. Med. Biol. 229: Chapter 9, pp. 107-130, 1988). LXA$_4$ has also been shown to induce neutrophils to generate superoxide radicals, release elastase, and promote chemotaxis by leukocytes (Serhan, C.N. et al., in Prostaglandins, Leukotrienes and Lipoxins, J.M. Bailey, ed., Plenum, New York, pp. 3-16, 1985).

It has been suggested to use LXA$_4$ to induce the inflammatory response of neutrophils so as to provide an experimental model to

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evaluate the efficacy of compounds such as LXB_4 derivatives in preventing this response (Samuelsson, B. et al., U.S. 4,560,514). It has also been suggested that LXA_4 may exert some of its biological effects by binding to the LTD_4 receptor (Jacques, C.A.J. et al., Br. J. Pharmacol. 95: 562-568 (1988)) and that LXA_4 and LTD_4 may even share a common receptor (Lefer, A.M. et al., Proc. Natl. Acad. Sci. USA 85:8340-8344 (1988)).

Slow-reacting substances of anaphylaxis (SRS-A) are considered to be the physiological mediators of anaphylactic and allergic reactions in animals. SRS-A consist primarily of a mixture of the LDE leukotrienes LTC_4 and LTD_4.

The release of SRS-A has also been implicated as the underlying cause of disorders of the mucociliary and cardiovascular system. SRS-A induced contraction of bronchial smooth muscle results in impairment of ventilatory function in allergic asthma. SRS-A induced impairment of mucus clearance leads to mucus plugging of the airways and bronchial hyper-reactivity seen in subjects with asthma. SRS-A induced pulmonary hypertension plays a role in cor polmonale, chronic bronchitis and emphysema. SRS-A induced negative inotropism, coronary vasoconstriction and increased vascular permeability have an effect on the cardiovascular system. SRS-A also participates in mediating the functional consequences of glomerular inflammatory injury (Badr, K.F., et al., J. Clin. Invest. 81:1702-1709 (1988)).

Inhibition of SRS-A, and ameliorations of the physiological consequences of SRS-A, can occur either by blocking release of SRS-A or by blocking the actions of released SRS-A. Thus inhibition of the actions of SRS-A may be used to alleviate and treat the above disorders.

It is known to inhibit physiological responses to SRS-A by blocking the LTD_4 receptor with LTD_4 receptor antagonists. Sheard et al. have described a synthetic compound, FPL 55712, and derivatives thereof, which inhibit the SRS-A response by acting as LTD_4 receptor antagonists (Sheard, P. et al., in The Development of Anti-Asthma

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<u>Drugs</u>, D.R. Buckle <u>et al</u>., eds., Butterworth, London, 1984, pp.133-158). However, FPL-55712 is short-acting. Other antagonists of SRS-A have been reported (for example, Gleason, J.G. <u>et al</u>., <u>J. Med. Chem.</u> <u>30</u>:959-961 (1987)) including many which retain close structural similarity with FPL 55712 (Fleisch, J.H., <u>et al</u>., <u>J. Pharmacol. Exp.</u> <u>Ther.</u> <u>233</u>:148 (1985); Young, R.N. <u>et al</u>., <u>J. Med. Chem.</u> <u>29</u>:1573 (1986); O'Donnell, M. <u>et al</u>., <u>Ann. Allergy</u> 278 (1985). However, most of these compounds exhibit a low affinity for LTD_4 receptors, are orally inactive or exhibit low bioavailability. The status of currently known LTD_4 receptor antagonists has been recently reviewed (Lefer, A.M., <u>ISI Atlas Sci. (Pharmacology)</u> <u>2</u>:109-115 (1988); Cashman, J.R. <u>et al</u>., <u>Drugs of Today</u> <u>24</u>:723-732 (1988); Fleisch, J.H. <u>et al</u>., <u>Ann. N. Y. Acad. Sci.</u> <u>524</u>:356-368 (1988); Musser, J.H. <u>et al</u>., <u>Agents and Actions</u> <u>18</u>:332-341 (1986)).

Studies in human neutrophils, have established an inverse relation between the generation of lipoxins and leukotrienes following exposure of these cells to 15-HETE and the calcium ionophore A23187 (Serhan, C.N., in <u>Advances in Prostaglandin, Thromboxane and Leukotriene Research</u>, Samuelsson, B., <u>et al</u>. (eds.), Raven Press, New York, Volume 18). In addition, recent results indicate that mesangial cells can generate lipoxins from exogenous sources of LTA_4 (Garrick, R., <u>et al</u>., <u>Kidney Int. Proceedings 21st Annual Meeting of the American Society of Nephrology</u>, Vol. 25, p. 292 (1989)), which may be provided by activated leukocytes (e.g., during transcellular metabolism). Thus, the local levels of these compounds may be elevated following the infiltration of leukocytes into the glomerulus.

Use of LTD_4 receptor antagonists which are based on a natural product would be preferable to the use of completely synthetic compounds in that the natural products would ameliorate the physiological consequences of leukotriene-induced physiological injury with greater bioavailability and less toxicity than the synthetic

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inhibitors. Thus it is desirable to develop SRS-A antagonists which are natural compounds or derivatives thereof.

Summary of the Invention

In response to the long-standing need for controlling the vasoconstriction associated with SRS-A, the present invention was developed. Recognizing that current compounds used as antagonists of the LTD_4 receptor were derived from a chemical structure unrelated to a natural product, the inventors postulated that fewer side effects and better bioavailability might be obtained with a natural antagonist of SRS-A or a compound structurally derived from a natural antagonist. The present invention is directed to compositions comprising natural antagonists of SRS-A, and especially of LTD_4. Further, the invention is directed to methods for antagonizing SRS-A, and especially LTD_4, in an animal, such methods comprising administration of antagonist-effective amounts of LXA_4 or an active derivative thereof to such animal. These antagonists and methods are useful in the treatment of physiological disorders in which LTD_4 receptors play a contributing role, for example, hemostasis, vascular reactivity, and especially vasoconstriction, inflammation, and anaphylactic and allergic reactions.

Brief Description of the Figures

Figure 1 is a plot of the percent inhibition of $[^3H]LTD_4$ (10 nM) binding to rat glomerular mesangial cells by LTD_4 (squares) and LXA_4 (triangles). Each point represents the mean of four experiments performed in duplicate.

Figure 2 is a plot of inositol triphosphate (IP_3) formation in rat mesangial cells in response to LXA_4 and LTD_4 and its complete abolition in the presence of 100-fold concentrations of SKF 104353 (SKF). The far-right column depicts the abrogation of LTD_4-induced

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stimulation of IP_3 formation by preexposure of the cells to 100 nM LXA_4 for 10 minutes. *, $\underline{P}<0.01$ versus vehicle-stimulated controls. +, $\underline{P},0.05$ versus LTD_4 alone.

Figure 3 is a plot of the percent reduction in glomerular filtration rate (GFR, panel A) and renal plasma flow (RPF, panel B) in response to increasing doses on intrarenal arterial LTD_4 without (filled squares) and with (open squares) LXA_4. See Table 1 for mean absolute values. *, $\underline{P},0.05$ versus corresponding points in the presence of LXA_4.

Definitions

In order to provide a clearer and consistent understanding of the specification and claims, including the scope to be given such terms, the following definitions are provided.

$\underline{LXA_4}$. By "LXA_4" is meant lipoxin A_4 or an active derivative thereof, including synthetic analogues of LXA_4 which are converted by the body to LXA_4 or an active derivative thereof, and pharmaceutically suitable salts thereof.

$\underline{Pharmaceutically\ Acceptable\ Salts.}$ By the term "pharmaceutically acceptable salts" is intended salts formed from pharmaceutically acceptable acids or bases, e.g., acids such as sulfuric, hydrochloric, nitric, phosphoric acid, etc. or bases such as alkali or alkaline earth metal hydroxides, ammonium hydroxides, alkyl ammonium hydroxides, etc.

$\underline{Animal.}$ By the term "animal" is intended all animals in which LXA_4 is capable of antagonizing LTD_4 receptor responses. Foremost among such animals are humans; however, the invention is not intended to be so limiting, it being within the contemplation of the present invention to treat any and all animals which may experience the beneficial effects of the invention.

$\underline{Receptor.}$ The term "receptor" is intended to refer generally to a functional macromolecule or complex of macromolecules with which

certain groups of cellular messengers, such as LDE's, hormones and neurotransmitters, must first interact before the biochemical and physiological responses to those messengers are initiated. Therefore, as used herein, the term "receptor" is used operationally to denote any cellular macromolecule (or complex of macromolecules) to which a chemical or macromolecular entity specifically binds to initiate its effects.

Agonist. The term "agonist" is intended to refer generally to a chemical agent which mimics at least some of the effects of a natural chemical messenger by interaction with the appropriate physiological receptor for the natural messenger.

Antagonist. The term "antagonist" is intended to refer generally to a chemical compound which is able to bind to the receptor for a natural chemical messenger, but which has no intrinsic physiological activity at that receptor and does not invoke the physiological response mediated by activation of that receptor. As recognized by those skilled in the art, the binding of an antagonist results in an interference with the effect of the natural ligand or with an agonist. As used herein, compounds that are themselves devoid of intrinsic pharmacological activity but cause effects by inhibition of the action of a specific messenger, by competition for receptor binding sites, are designated as antagonists. Thus, a method for "antagonizing" a response to specific agents such as SRS-A is a method in which the natural binding of SRS-A to specific SRS-A receptors is interferred with so as to block the activation of such receptors.

Response. The term "response" is intended to refer to a change in any parameter which can be used to measure and describe an effect associated with interaction of a chemical agent with the LTD_4 receptor. The response may be a physical one such as a change in the vascular reactivity, renal glomerular dynamics or pulmonary functioning; or, it may be a molecular one for example, a change in a level of a metabolite, (for example, IP_3) or protein, receptor, enzyme, or genomic expression.

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Controlling.   By "controlling" a response, for example,
controlling vasoconstriction, is mean ameliorating, reversing,
regulating or interfering with such response, wherein such
amelioration, reversal, regulation or interference is the result of
administration of anti-response effective amounts of a desired agent.
Thus, administration of anti-vasoconstrictive effective amounts of an
anti-vasoconstriction agent results in amelioration, reversal,
regulation or interference with vasoconstriction. Such administration
may be used to medically "treat" the response in an animal so as to
improve a medical disorder, disease or symptom associated with the
response.

Agent.   The term "agent," for example a anti-vasoconstriction
agent, is intended to refer generically to any compound which
interacts with the stated receptor or response.

Detailed Description of the Invention

The present invention comprises methods of treating leukotriene-
induced vasoconstriction by administration of efficacious levels of
LXA_4 and derivatives thereof. The compounds of the invention are LTD_4
receptor antagonists and inhibit LTD_4 receptor action; this makes the
compounds useful in the treatment and prevention of disorders and
disease states wherein leukotriene-induced vasoconstriction is
implicated as a contributing or causative agent such as, but not
limited to, disorders resulting from the release of SRS-A, and
especially asthma, anaphylactic reactions, allergic reactions, shock,
circulatory disease states and inflammatory reactions such as, for
example, rheumatoid arthritis, gout, psoriasis, allergic rhinitis,
adult respiratory distress syndrome, Crohn's disease, endotoxin shock,
traumatic shock, hemorrhagic shock, bowel ischemic shock (i.e.
splanchnic artery occlusion shock), renal glomerular disease, benign
prostatic hypertrophy, inflammatory bowel disease, and/or myocardial
ischemia and infarction, circulatory shock, brain injury, systemic

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lupus erythematosus, and hypertension, especially essential hypertension and malignant hypertension.

The methods of the invention are based on the inventors' discovery that selective administration of LXA_4 into the renal artery of anesthetized rats elicits vasodilator responses in renal arterioles, and reduces the glomerular capillary ultrafiltration coefficient (K_f) (Badr, K.F., et al., Am. J. Physiol. 22:F239-F243 (1987); Badr, K.F., et al., Biochem. Biophys. Res. Comm. 145:408-414 (1987)). The latter results from the concerted contractile action of smooth muscle-containing glomerular mesangial cells which reduces the glomerular capillary area available for ultrafiltration and, hence, K_f. Since the orientation of the polar groups is similar in the peptido-LTs and LXA_4 (i.e., 5S,6R) and is a crucial requirement for biologic activity of these eicosanoids, the inventors investigated whether LXA_4-induced falls in K_f were due, in part, to interaction with the mesangial cell LTD_4 receptor. Surprisingly, the inventors discovered that LXA_4 was capable of binding to the mesagial cell LTD_4 receptor and of acting as a LTD_4 receptor antagonist. The inventors realized that LXA_4 would function as a LTD_4 receptor antagonist in any tissue sensitive to LTD_4 action. Such a finding is a significant advance in the treatment of the functional consequences of glomerular inflammatory reactions (Badr, K.F., et al., J. Clin. Invest. 81:1702-1709 (1988); Badr, K.F., in Advances in Prostaglandin, Thromboxane and Leukotriene Research, Samuelsson, B., et al. (eds.), Raven Press, New York, Volume 18 (1989); and Badr, K.R. et al., Proc. Natl. Acad. Sci. USA 86: (1989), incorporated herein by reference)).

LXA_4 can be isolated from leukocytes exposed to 15-HPETE ((15S), 15-hydroperoxy-5,8,11-cis-13-trans-eicosatetraenoic acid (Serhan, C.N. et al., J. Biol. Chem. 261:16340-16345 (1986)), incorporated herein by reference, or chemically synthesized (Webber, S. E. et al., Adv. Exper. Med. Biol. 229:61-78 in Lipoxins: Biosynthesis, Chemistry, and Biological Activities, P. Wong et al., eds., Plenum Publishing Corporation, New York, 1988).

As will be understood by one of skill in the art, derivatives of LXA_4 may be constructed which, while retaining the ability of the natural compound to act as a LTD_4 receptor antagonist, have an enhanced bioavailability, half-life, affinity for the LTD_4 receptor, or other desirable property. Such derivatives may include covalent substitutions at one or more of the three hydroxyl groups and/or at the terminal carboxyl group of LXA_4. Especially, such substitutents include esterification of the carboxyl group, and preferably, the α-methyl ester derivative thereof. Substitutents at carbon positions 5, 6, and 15 include addition of the acetate, methyl, of n-butylboronate derivative.

LXA_4 and derivatives thereof can be administered by any method which results in deliverance of efficacious levels of said LXA_4 or derivatives thereof such that amelioration of the targeted leukotriene-based physiological disorder occurs. For example, LXA_4 may be administered parenterally, orally, or topically, in dosage unit formulations containing conventional nontoxic pharmaceutically acceptable carriers, adjuvants and vehicles as desired. The term parenteral as used herein includes subcutaneous, intravenous, intraarterial injection or infusion techniques, without limitation. The term "topically" encompasses administration rectally and by inhalation spray (aerosol), as well as by the more common routes of the skin and the mucous membrane of the mouth and nose.

Total daily doses of the compounds of this invention administered to a host in single or divided doses may be in amounts, for example, of from about 0.1 to about 10 mg/kg body weight daily and more preferrably 0.5 to 5 mg/kg/day. Dosage unit composition may contain such amounts of such submultiples thereof as may be used to make up the daily doses. It will be understood however, that the specific dose level for any particular patient will depend upon a variety of factors including the body weight, general health, sex, diet, time and routine of administration, rates of absorption and excretion,

combination with other drugs and the severity of the particular disease being treated.

This invention also provides for compositions in unit dosage form for the suppression or inhibition of LTD_4 receptor activity in a human or lower animal host in need of such treatment, comprising LXA_4 or an active derivative thereof and one or more nontoxic pharmaceutically acceptable carriers, adjuvants, or vehicles. The amount of active ingredients that may be combined with such materials to produce a single dosage form will vary depending upon various factors, as indicated above. As described herein, variety of materials can be used as carriers, adjuvants and vehicles in the composition of this invention, as available in the pharmaceutical arts.

Injectable preparations, such as oleaginous solutions, suspensions or emulsions, may be formulated according to known art, using suitable dispersing or wetting agents and suspending agents, as needed. When the active compounds are in water-soluble form, for example, in the form of water soluble salts, the sterile injectable preparation may employ a nontoxic parenterally acceptable diluent or solvent as, for example, sterile nonpyrogenic water or 1,3-butanediol. Among the other acceptable vehicles and solvents that may be employed are 5% dextrose injection, Ringer's injection and isotonic sodium chloride injection (as described in the USP/NF). When the active compounds are in a non-water soluble form, sterile, appropriate oily suspensions containing suitable lipophilic solvents or vehicles, such as fatty oil, for example, sesame oil, or synthetic fatty acid esters, for example, ethyl oleate or triglycerides, are used. Alternatively, aqueous injection suspensions which contain substances which increase the viscosity, for example, sodium carboxymethyl cellulose, sorbitol, and/or dextran, and optionally also contain stabilizers may be used.

The pharmaceutical preparations of the present invention are manufactured in a manner which is in itself know, for example, by means of conventional mixing, granulating, dragee-making, dissolving, or lyophilizing processes. Thus, pharmaceutical preparations for oral

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use can be obtained by combining the active compounds with solid excipients, optionally granulating a resulting mixture and processing the mixture or granules, after adding suitable auxiliaries, if desired or necessary, to give tablets of dragee cores.

Suitable excipients are, in particular, fillers such as sugars, for example lactose or sucrose, mannitol or sorbitol, cellulose preparations and/or calcium phosphates, for example tricalcium phosphate or calcium hydrogen phosphate, as well as binders, such as starch, pastes, using, for example, maize starch, wheat starch, rice starch, or potato starch, gelatine, tragacanth, methyl cellulose, hydroxypropylmethyl cellulose, sodium carboxymethyl cellulose, and/or polyvinyl pyrrolidone, and/or, if desired, disintegrating agents, such as the above-mentioned starches, and also carboxymethyl-starch, cross-linked polyvinyl pyrrolidone, agar or alginic acid or a salt thereof, such as sodium alginate. Auxiliaries are, above all, flow-regulating agents and lubricants, for example, silica, talc, stearic acid or salts thereof, such as magnesium stearate or calcium stearate, with suitable coating, which if desired, are resistant to gastric juices and for this purpose, inter alia concentrated sugar solutions, which optionally contain gum arabic, talc, polyvinyl pyrrolidone, polyethylene glycol and/or titanium dioxide, lacquer solutions and suitable organic solvents or solvent mixtures. In order to produce coatings resistant to gastric juices, solutions of suitable cellulose preparations such as acetyl cellulose phthalate or hydroxypropylmethyl cellulose phthalate, are used. Dyestuffs or pigments may be added to the tablets or dragee coatings, for example, for identification or in order to characterize different combinations of active compound doses.

Other pharmaceutical preparations which can be used orally are push-fit capsules made of gelatin, as well as soft, sealed capsules made of gelatin and a plasticizer such as glycerol or sorbitol. The push-fit capsules can contain the active compounds in the form of granules, for example, mixed with fillers such as lactose, binders such as starches, and/or lubricants such as talc or magnesium stearate

and, optionally, stabilizers. In soft capsules, the active compounds are preferably dissolved or suspended in suitable liquids, such as fatty oils, liquid paraffin or liquid polyethylene glycols, it also being possible to add stabilizers.

Suppositories for rectal administration of the compound of this invention can be prepared by mixing the drug with suitable suppository bases such as a nonirritating excipient, for example, cocoa butter, natural or synthetic triglycerides, paraffin hydrocarbons, polyethylene glycols, or higher alkanols, and especially bases which are solid at ordinary temperature but liquid at body temperature and which therefore melt in the rectum and release the drug. In addition, it is possible to use gelatin rectal capsules which consist of a combination of the active compounds with a base; possible base materials are, for example, liquid triglycerides, polyethylene glycols, or paraffin hydrocarbons.

Solid dosage forms for oral administration include capsules, tablets, pills, troches, lozenges, powders and granules. In such solid dosage forms, the active compound may be admixed with at least one inert diluent such as sucrose, lactose or starch. Such dosage forms may also comprise, as is normal practice, pharmaceutical adjuvant substances, e.g., stearate lubricating agents. Solid oral preparations can also be prepared with enteric or other coatings which modulate release of the active ingredients.

Liquid dosage forms for oral administration include pharmaceutically acceptable emulsions, solutions, suspensions, syrups and elixirs containing inert nontoxic diluents commonly used in the art, such as water and alcohol. Such compositions may also comprise adjuvants, such as wetting agents, emulsifying, suspending, sweetening, flavoring and perfuming agents.

The compositions of the present invention, in and of themselves, find utility in the control of vasoconstriction, be it chronic or acute. The compositions for the present invention direct the body's own mechanisms for dealing with vasoconstriction to its maximum

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potential.   In intravenous dosage form, the compositions of the
present invention have a sufficiently rapid onset of action to be
useful in the management of vasoconstriction of short duration such as
that found in vasospastic responses or malignant hypertension.

Additionally, a low potency version is useful in the management
of mild or chronic vasoconstriction.   This low potency version is
useful in the management of essential hypertension, and chronic renal
disease.

Further, it has also been found that the compositions of the
present invention are useful in the management of chronic severe
vasoconstriction such that associated with cardiovascular disease and
glomerular kidney disease.

In addition, the compounds of the present invention provides
insight into the structural requirements for leukotriene receptor
activation and show that lipoxins can serve as structural models for
the design of more potent receptor level antagonists for the
peptidoleukotrienes.

The following examples are illustrative, but not limitative of
the method and composition of the present invention.   Other suitable
modifications and adaptations of the variety of conditions and
parameters normally encountered in clinical therapy and which are
obvious to those skilled in the art are within the spirit and scope of
this invention.

EXAMPLES

Statistical: ANOVA with Bonferroni modification for multiple
pre-planned comparisons was used, within each group and between
groups, to compare the changes in various whole kidney and micro-
circulatory indices which occurred from one period to another.   In
phosphoinositide generation studies, increases in $[^3H]$-IP_3 CPMs in

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agonist-treated cells were compared to vehicle-treated controls using unpaired students t-test. Differences were considered significant at a p value ≤ 0.05. All values are reported as mean $\pm$ SEM.

Example 1
Binding of LXA₄ to LTD₄ Receptors In Kidney Mesangial Cells

The ability of LXA$_4$ to bind to mesangial cell LTD$_4$ receptors in a concentration-dependent manner was investigated in mesangial cell cultures.

Mesangial Cell Culture: Rat mesangial cells were isolated and cultured as described previously (Harris, R.C., et al., J. Clin. Invest. 82:1028-1039 (1988)). Briefly, kidneys were excised from two young Sprague-Dawley rats under sterile conditions, and the cortices removed, minced, and washed several times in Hank's Balanced Salt Solution, containing 10 mM HEPES pH 7.4, amphotericin (0.25 μg/ml), and gentamicin (50 μg/ml). The tissue was then passed through consecutive sterilized stainless mesh filters with pore sizes of 212 μm, 150 μm, and then onto the final mesh of 75 μm. The isolated glomeruli were harvested from the surface of the 75 μm filter and washed twice with the Hank's Media. The glomeruli were suspended in RPMI 1640 Medium with 15% Fetal Bovine Serum (Gibco), penicillin (100 U/ml) and streptomycin (100 μg/ml) (Gibco), plated into 100 mm cell culture petri dishes, and incubated at 37°C in a humidified atmosphere of 95% air and 5% CO_2. Mesangial cell colonies were subcultured in 60 mm culture dishes, and experiments were carried out in cells from passages 2 to 12. The criteria used to establish the identity of mesangial cells were as described previously (Harris, R.C., et al., J. Clin. Invest. 82:1028-1039 (1988)). Cells were routinely grown in RPMI-1640, supplemented with 20% fetal calf serum, penicillin 100 U/ml, and streptomycin 100 μg/ml.

[³H]-LTD₄ Binding Studies: LXA$_4$ was obtained from Biomol Research Laboratories Inc., Philadelphia, PA and [14,15-³H] LTD$_4$ (32.0 Ci/mmol) was obtained from New England Nuclear. All eicosanoids were

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stored under argon at -70°C in methanol and their purity and quantity verified periodically by ultraviolet scanning and high pressure liquid chromatography (Serhan, C.N. et al., J. Biol. Chem. 261:16340 (1986)).

Studies of [3H]-LTD$_4$ binding were performed on mesangial cells grown to confluence in 24-well cluster dishes. Preliminary experiments indicated that increased [3H]-LTD$_4$ binding in other systems (Lewis, M.A., et al., Biochem. Pharmacol. 34:4311-4317 (1985); Sarau, H.M., et al., J. Biol. Chem. 262:4034-4041 (1987)). Therefore, experiments were performed in a buffer previously determined to optimize [3H]-LTD$_4$ binding (Sarau, H.M., et al., J. Biol. Chem. 262:4034-4041 (1987); Mong, S., et al., Mol. Pharmacol. 32:223-229 (1987)) consisting of 20 mM HEPES, 10 mM CaCl$_2$, 10 mM MgCl$_2$, 5 mM glycine and 5 mM cysteine (Buffer A). Binding studies were routinely performed at 4°C. Cells were washed once with buffer A and then exposed to the appropriate concentration of [3H]-LTD$_4$ in buffer A. At the completion of the experiment, the experimental medium was removed and the cells were washed 5 times with ice-cold buffer. The cells were then dissolved with 1.0 ml of 1.0 N NaOH, neutralized with HCl, dissolved in 10 ml of Aquasol (New England Nuclear, Boston, MA), and the bound radioactivity was determined using a scintillation counter (Beckman Instruments). Non-specific binding was determined by measuring the amount of [3H]-LTD$_4$ bound in the presence of 1000-fold excess of unlabeled LTD$_4$. Cell density was determined by counting cells from replicate wells, using a Coulter Counter ZBi (Coulter Electronics, Inc., Hialeah, FL).

Competitive binding-inhibition studies were carried out by incubation of mesangial cells with 10 nM [3H]-LTD$_4$ and addition, at equilibrium, of 10-fold to 1000-fold increasing concentrations of LXA$_4$.

Figure 1 depicts the percent inhibition of the binding of 10 nm [3H]-LTD$_4$ by LTD$_4$ and LXA$_4$. In early passage cultured mesangial cells, LXA$_4$ was a potent competitor for [3H]-LTD$_4$ binding in a manner similar to that of unlabeled LTD$_4$ (Figure 1). Half maximal inhibition

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of binding by LXA$_4$ was at 100 nM compared to 100 nM for the homoligand.

Competition by LXA$_4$ was equipotent to that of LTE$_4$ and several-fold greater than that of the non-peptidoleukotriene, LTB$_4$. The potent [^{3}H]-LTD$_4$ binding inhibition obtained with LXA$_4$, compared to the relatively weak competitive properties of the biologically inactive 5R,6S-LTD$_4$ isomer, suggests strongly that the S,R orientation of the polar substituents at C5 and C6 confers optimal advantage for receptor recognition and biological activity. This is supported by additional studies in which 6S-LXA$_4$ (5S,6S,15S,trihydroxy-7,9,13-trans-11-cis-eicosatetraenoic acid) failed to compete for [^{3}H]-LTD$_4$ even at a 1000-fold excess concentration.

These results demonstrate that the binding of [^{3}H]-LTD$_4$ to rat mesangial cells displays a number of characteristics which suggest the presence of specific, membrane-bound receptors for this eicosanoid on mesangial cells. The presence of such receptors takes on particular importance in view of the demonstrated functional responses of mesangial cells to exogenous LTD$_4$ and their possible relevance during inflammatory glomerular injury: LTD$_4$ contracts mesangial cells in culture (Barnett, R., et al., Am. J. Physiol. 19:F838-F844 (1986); Simonson, M.S., et al., Kidney Int. 30:524-531 (1986)), markedly reduces K$_f$ and GFR in vivo (Badr, K.F., et al., Am. J. Physiol. 22:F239-F243 (1987); Badr, K.F., et al., Circ. Res. 54:492-499 (1984)), and, through these actions, plays an important role in mediating the impairment of glomerular filtration and permselectivity functions in experimental models of glomerulonephritis (Badr, K.F., et al., J. Clin. Invest. 81:1702-1709 (1988)).

Example 2
Inhibition of LTD$_4$-Induced IP$_3$ Formation by LXA$_4$

LXA$_4$ also blocked LTD$_4$-induced stimulation of IP$_3$ formation (Figure 2). To label mesangial cells with [^{3}H]-Inositol, cells grown to confluence in 60 mm culture dishes (10^6 cells/dish) were cultured

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in RPMI, supplemented with 10% dialyzed fetal calf serum and $[^3H]$-inositol (1 μCi/ml) in a total incubation volume of 5 ml. Preliminary experiments revealed that incorporation of label was maximal at 48 hours and stable for up to 72 hours. All subsequent experiments were therefore carried out on cells preincubated with $[^3H$-inositol for 48-60 hours.

The formation of inositol monophosphate (IP_1), inositol bisphosphate (IP_2), and IP_3 in $[^3H]$-inositol labeled mesangial cells in response to either LTD_4 or LXA_4 was measured as follows: plates were washed with Krebs-Ringer solution containing 118 mM NaCl, 4.6 mM KCl, 24.9 mM $NaHCO_3$, 1 mM KH_2PO_4, 11.1 mM glucose, 1.1 mM $MgSO_4$, 1.0 mM CaCl, 5 mM HEPES, and 0.1% BSA, pH 7.4, 37°C. Increasing concentrations (0.1 to 100 nM) of LTD_4, LXA_4, or LTD_4 following preincubation of cells with 100-fold concentration LXA_4 for 10 min were then added to 2 ml of warmed (37°C) Krebs-Ringer and the cells incubated for the indicated time. In experiments in which the effect of the LTD_4 antagonist, SKF 104353, on IP formation was tested, the cells were preincuated in the presence of this agent for 10 min prior to addition of agonist (Mong, S., et al., Mol. Pharmacol. 32:223-229 (1987); Gleason, J.G., et al., J. Med. Chem. 30:959-961 (1987)). Reactions were terminated by the addition of cold 10% trichloroacetic acid (TCA), the supernatants subjected to ether extraction, and the aqueous phase titrated to neutral pH using 0.1 M Tris base. $[^3H]$-IP_1, $[^3H]$-IP_2, and $[^3H]$-IP_3 in the aqueous phase were separated and quantitated by anion exchange column chromatography, using the method of Berridge et al. (Berridge, M.J., Biochem. J. 220:345-360 (1984)). To ensure the reliability of the separation technique, the same methods for extraction and separation were applied to known amounts of $[^3H]$-IP_1, $[^3H]$-IP_2, and $[^3H]$-IP_3 standards (New England Nuclear) mixed in a ratio of 3:2:1, respectively, and subjected to separation by HPLC. The latter was performed using established methodology described previously (Cunha-Melo, J.R., et al., J. Biol. Chem. 262:11455-11463 (1987)). The results from both separation techniques

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revealed indistinguishable differences in the percentage of recovered counts (about 80%) and the quantitative ratio of the three IPs separated.

Phosphoinositide hydrolysis in mesangial cells was measured five secs after addition of 1 nM (n = 3), 10 nM (n = 6), 50 nM (n = 3), and 100 nM (n = 5) LXA_4 to mesangial cells labeled with $[^3H]$-inositol. Low but significant increases [46±14% (p < 0.05), 50±21% (p<0.05), 44±22% (p<0.05), and 45±26% (p<0.05), respectively] in radioactive counts (CPM) corresponding to IP_3 formation by these cells was noted over those obtained in vehicle-treated samples (controls). Addition of 10 nM (n=5) and 50 nM (n=4) LTD_4 to these cells was associated with 146±20% (p<0.01) and 106±13% (p<0.005) increases in $[^3H]$-IP_3 counts as compared to controls, values significantly greater than those for LXA_4 (p<0.05). Incubation of the mesangial cells with the LTD_4 receptor antagonist, SKF 104353, in concentrations 100-fold in excess of the ligand, followed by addition of LTD_4 in the 10 and 50 nM concentrations (n=3 for each) was associated with total abrogation of the LTD_4-induced stimulation of $[^3H]$-IP_3 formation in these cells. Similarly, pre-incubation of these cells with 100 nM LXA_4 for 10 min prior to addition of 10 nM LTD_4 (n=3) completely prevented LTD_4-induced IP_3 generation. Incubation of mesangial cells with SKF 104353 (100 nM) also abrogated the stimulation of IP_3 formation induced by LXA_4 (n=3) (Figure 2).

Intracellular generation of IP_3 is, in general, held to play an important role in mediating responses to various vasoactive agents. The demonstration of a rapid (5 sec) formation of IP_3 following addition of LTD_4 to mesangial cells is in agreement with reports by other investigators regarding the involvement of phosphoinositide hydrolysis in the signaling of LTD_4-mediated biological responses in rat basophilic leukemia cells (Sarau, H.M., et al., J. Biol. Chem. 262:4034-4041 (1987)), sheep tracheal smooth muscle cells (Mong, S., et al., J. Pharmacol. Exp. Ther. 244: 508-515 (1988)), bovine endothelial cells (Clark, M.A., et al., Proc. Natl. Acad. Sci. USA

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83:7320-7324 (1986)), and guinea pig lung (Mong, S., _et al._, _Mol._ _Pharmacol._ 31:35-41 (1987)). It is reasonable to assume that the contraction of mesangial cells by LTD_4 may proceed via a similar mechanism involving the formation of IP_3 and mobilization of intracellular Ca^{2+}. In fact, the capacity of LTD_4 to increase intracellular Ca^{2+} concentrations has been demonstrated recently by Baud _et al._ in DMSO-differentiated HL-60 myeloid cells (Baud, L., _et_ _al._, _J. Clin. Invest._ 80:983-991 (1987)). In the present studies, LXA_4 elicited similar, though significantly less potent stimulation of IP_3 generation in mesangial cells, presumably due to occupancy of the LTD_4 receptor. That LXA_4-induced IP_3 formation is indeed a consequence of LTD_4 receptor activation is supported by its total abrogation in the presence of a specific LTD_4 receptor antagonist, SKF 104353 (Figure 2). SKF 104353 is a structural analog of LTD_4 and LTE_4 that itself is devoid of agonist activity both _in vivo_ (Badr, K.F., _et_ _al._, _Am. J. Physiol._ 22:F239-F243 (1987); Mong, S., _et al._, _Mol._ _Pharmacol._ 32:223-229 (1987)) and _in vitro_ (Mong, S., _et al._, _Mol._ _Pharmacol._ 32:223-229 (1987); Gleason, J.G., _et al._, _J. Med. Chem._ 30:959-961 (1987)), and it competes for the binding of homoligands to LTD_4/LTE_4 receptors in a highly potent and specific manner (Gleason, J.G., _et al._, _J. Med. Chem._ 30:959-961 (1987)), 400- to 500-fold more effectively than the putative leukotriene antagonist, FPL 55712 (Gleason, J.G., _et al._, _J. Med. Chem._ 30:959-961 (1987)). It is capable of antagonizing and reversing LTD_4 effects in a number of tissues, including human and guinea pig lung (Mong, S., _et al._, _Mol._ _Pharmacol._ 32:223-229 (1987)), rat basophilic leukemia cells (Sarau, H.M., _et al._, _J. Biol. Chem._ 262:4034-4041 (1987)), and renal vasculature (Badr, K.F., _et al._, _J. Clin. Invest._ 81:1702-1709 (1988)). In systems where it has been tested, SKF 104353 was found to compete specifically with [^{3}H]-LTD_4 for binding to whole cells (Sarau, H.M., _et al._, _J. Biol. Chem._ 262:4034-4041 (1987); Mong, S., _et al._, _Mol. Pharmacol._ 32:223-229 (1987); Gleason, J.G., _et al._, _J. Med._ _Chem._ 30:959-961 (1987)), as well as cell membrane fractions (Sarau,

H.M., et al., J. Biol. Chem. 262:4034-4041 (1987); Mong, S., et al., Mol. Pharmacol. 32:223-229 (1987); Gleason, J.G., et al., J. Med. Chem. 30:959-961 (1987)), and it shifts the dose-response curves obtained for LTD_4-mediated phosphoinositide hydrolysis, intracellular calcium mobilization, and thromboxane synthesis (Sarau, H.M., et al., J. Biol. Chem. 262:4034-4041 (1987); Mong, S., et al., Mol. Pharmacol. 32:223-229 (1987); Gleason, J.G., et al., J. Med. Chem. 30:959-961 (1987)). In other studies, we demonstrated the capacity of this antagonist to inhibit [^{3}H]-LTD_4 binding to its mesangial cell binding site in a manner similar to that reported for this compound in other systems (Sarau, H.M., et al., J. Biol. Chem. 262:4034-4041 (1987); Mong, S., et al., Mol. Pharmacol. 32:223-229 (1987); Gleason, J.G., et al., J. Med. Chem. 30:959-961 (1987)). When taken together, the findings that LXA_4 (1) blocks the binding of [^{3}H]-LTD_4, (2) effects the generation of IP_3, and (3) is antagonized by a well-defined LTD_4 receptor antagonist (e.g., SKF 104353) establish the involvement of a common recognition site in mediating the actions of these two eicosanoids on mesangial cells.

Example 3
Physiologic Interactions of LTD_4 and LXA_4

Renal function was measured in vivo in Inactin-anesthetized male Munich-Wistar rats weighing 220-240 gms which were surgically prepared according to protocols described previously (Badr, K.F., et al., Am. J. Physiol. 22:F239-F243 (1987)). Inulin and para-aminohippurate (PAH) clearances were used to measure GFR and effective RPF. A needle was placed at the take-off of the left renal artery through which a maintenance infusion of 0.9% NaCl at a rate of 0.05 ml/min was initiated. This infusion originated from two separate pumps each delivering at 0.025 ml/min and connected to the renal arterial catheter by means of a three-way connector. Homologous rat plasma was administered intravenously according to a protocol shown previously to maintain euvolemia (Badr, K.F., et al., Am. J. Physiol. 22:F239-F243

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(1987)). Intra-renal arterial administration of LTD4 (1 μg/kg/min) results in systemic hemodynamic alterations identical to those we previously reported for LTC_4 (Badr, K.F., et al., Am. J. Physiol. 22:F239-F243 (1987); Badr, K.F., et al., Circ. Res. 54:492-499 (1984)) which included an elevation in systemic arterial pressure and a progressive loss of plasma volume. In order to minimize the systemic effects of LTD_4 and allow for adequate assessment of renal functional parameters, the rate of infusion of homologous rat plasma was increased upon starting LTD_4 and approximately 4-5 ml of plasma were administered throughout the LT infusion. The degree of change in hematocrit varied with the dose of LTD_4 (Table 1). Inulin and PAH concentrations were determined according to Fuhr et al. (Führ, J., Klin. Wochhenschr. 33:729-499 (1955)) and Smith et al. (Smith, H.W., et al., J. Clin. Invest. 24:388-391 (1945)). Two experimental groups were studied as follows:

Group I (n = 14). First period: Clearances were performed during vehicle infusion. Second period: Measurements were repeated during a 20-minute infusion of LTD_4 delivered through one of the infusion pumps into the left renal artery at doses of 0.5 (n = 4), 7 (n = 3), 14 (n = 3), and 20 (n = 4) μg/kg/min.

Group II (n = 12). First period: Clearances were performed during vehicle infusion. Second period: Measurements were repeated during a 20-min infusion of LXA_4 as in the second period, LTD_4 infusion was initiated through the second pump again in doses of 0.5 (n = 3), 7 (n = 3), 14 (n = 3), and 20 (n = 3) μg/kg/min (as in Group I).

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TABLE 1

		AP	Hct	RPF	GFR
		(mmHg)	(Vol./dl)	------ml/min------	
			GROUP I		
CONTROL	A (n=4)	118±8	46.3±0.7	4.35±0.17	1.11±0.02
[Period 1]	B (n=3)	110±5	45.3±2.2	4.53±0.13	1.15±0.10
	C (n=3)	124±3	49.4±1.0	4.33±0.12	1.08±0.03
	D (n=4)	112±4	46.1±0.8	4.45±0.08	1.12±0.04
LTD$_4$	A	127±9*	48.3±1.2	3.41±0.50	0.81±0.11
[Period 2]	B	124±6*	53.0±1.5*	2.76±0.59*	0.84±0.15*
	C	148±5*	59.2±2.2*	1.51±0.44*	0.32±0.10*
	D	128±4*	55.0±1.8*	1.20±0.15*	0.34±0.80*
			GROUP II		
CONTROL	A (n=3)	116±6	43.7±0.9	4.37±0.04	1.02±0.05
[Period 1]	B (n=3)	115±1	45.3±0.9	4.29±1.09	0.94±0.10
	C (n=3)	129±3	44.0±0.7	4.15±0.30	1.00±0.20
	D (n=3)	110±2	45.2±0.1	3.98±0.45	1.08±0.05
LXA$_4$	A	121±4	43.7±0.9	4.50±0.08	1.20±0.20
[Period 2]	B	120±0	44.7±1.2	4.76±0.83*	0.98±0.06
	C	127±2	45.2±0.7	4.87±0.10*	1.30±0.10*
	D	108±3	44.5±0.3	4.58±0.20*	1.27±0.04*
LXA$_4$+LTD$_4$	A	126±3	44.3±2.6	3.60±0.43†	1.08±0.15
[Period 3]	B	130±4†	50.3±0.7†	2.94±0.44†	0.88±0.18
	C	147±7†	51.2±1.0†	2.40±0.32†	0.74±0.20†
	D	131±3†	54.0±1.7†	1.58±0.30†	0.65±0.10†

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Administration of LTD_4 [LTD_4 doses: 0.5, 7.0, 14.0, and 20.0 (μg/kg/min)] in Group I animals was associated with dose-dependent reductions in the mean value for GFR and RPF which are summarized in Table 1.   In Group II animals, administration of LXA_4 was associated with moderate increases in both GFR and RPF in all animals (Table 1). These responses to LTD_4 and LXA_4 were as expected from previously reported studies (Badr, K.F., et al., Am. J. Physiol. 22:F239-F243 (1987); Badr, K.F., et al., Biochem. Biophys. Res. Comm. 145:408-414 (1987); Badr, K.F., et al., Circ. Res. 54:492-499 (1984)).   The administration of increasing doses of LTD_4 in the presence of LXA_4, however (Group II, third period), was associated with marked blunting of the LTD_4-provoked reduction in GFR (period 2 as compared to period 3), but was without effect on OTD_4-induced falls in RPF (Table 1). Mean percent falls in GFR/RPF during LTD_4 administration were 27*/24, 25*/40*, 70*/65*, and 73*/70* at the above doses, respectively (* $p<0.05$ vs baseline).   In the presence of LXA_4, these values were: 9/20*, 11/37*, and 42*/51*, and 50*/68*.   The dose-response curves for LTD_4-induced falls in GFR and RPF in the absence and presence of LXA_4 are depicted in Figure 3.

In   previous   studies   utilizing   micropuncture   techniques,   we defined the glomerular microcirculatory responses to both LTD_4 (Badr, K.F., et al., Am. J. Physiol. 22:F239-F243 (1987)) and LXA_4 (Badr, K.F., et al., Biochem. Biophys. Res. Comm. 145:408-414 (1987)) in the Munich-Wistar rat.   While LTD_4 is a renal vasoconstrictor, the principal mechanism through which it leads to a reduction in GFR is its potent K_f-lowering action, resulting from mesangial cell contrac- tion (Badr, K.F., et al., Am. J. Physiol. 22:F239-F243 (1987)).   These effects of LTD_4 on glomerular perfusion and function are evidenced by the dose-response curves established for this eicosanoid in Group I rats (Figure 3).   The vasodilatory and GFR-augmenting effects of LXA_4 (Badr, K.F., et al., Biochem. Biophys. Res. Comm. 145:408-414 (1987)), on the other hand, are evident in the changes in GFR and RPF seen in

Group II animals (Period 2 vs. Period 1). The subsequent administration of LTD_4 in these rats during the continued infusion of LXA_4, however, is associated with a dramatic shift in the dose-response for the LTD_4-induced falls in GFR (Period 2 to Period 3 in Group 2 rats). The relative protection against the GFR-depressant action of LTD_4 (which is provided by LXA_4), despite absence of any modification of LTD_4's potent vasoconstrictor action (Figure 3B), is interpreted as LXA_4-mediated prevention of LTD_4-induced fall in K_f. Although it is conceivable that the afferent arteriolar dilatory action of LXA_4 may have augmented intraglomerular capillary pressure (P_{GC}), and hence increased GFR, the elevated values of P_{GC} observed during LTD_4 infusion (Badr, K.F., et al., Am. J. Physiol. 22:F239-F243 (1987)) argues against further elevations in this parameter as a mechanism for the observed preservation of GFR. It is possible, however, that as yet undefined actions of LXA_4 may play some role in the in vivo experiments. Nevertheless, these in vivo observations lend strong support to our in vitro studies demonstrating competition for LXA_4 for the LTD_4 mesangial cell receptor and its inhibition of LTD_4-induced IP_3 formation in these cells. They also provide a basis for possible relevance of LT/LX interactions in the regulation of glomerular function, particularly during inflammatory injury (Badr, K.F., et al., J. Clin. Invest. 81:1702-1709 (1988)).

In summary, we have shown that LXA_4 can displace LTD_4 from its binding site on rat glomerular mesangial cells and block LTD_4-induced IP_3 formation, as well as offset the physiological contractile actions of LTD_4 on these cells in vivo. Together these findings suggest that LXA_4, a product of human leukocytes, may regulate the actions of the peptidoleukotrienes in vivo.

Having now fully described this invention, it will be understood by those with skill in the art that the invention may be performed

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within a wide and equivalent range of conditions, parameters, and the like without affecting the spirit or scope of the invention or any embodiment thereof.

WHAT IS CLAIMED IS:

1.   A method for antagonizing a response to SRS-A in an animal comprising administering a composition comprising an SRS-A-antagonist effective amount of LXA$_4$ or an active derivative thereof to said animal.

2.   The method of claim 1, wherein said SRS-A is a leukotriene.

3.   The method of claim 2, wherein said leukotriene is LTD$_4$.

4.   The method of claim 1, wherein said response to SRS-A is vasoconstriction.

5.   The method of claim 1, wherein said response to SRS-A is associated with a medical disorder selected from the group consisting of asthma, anaphylactic reactions, allergic reactions, shock, inflammation, rheumatoid arthritis, gout, psoriasis, allergic rhinitis, adult respiratory distress syndrome, Crohn's disease, endotoxin shock, traumatic shock, hemorrhagic shock, bowel ischemic shock, renal glomerular disease, benign prostatic hypertrophy, inflammatory bowel disease, myocardial ischemia, myocardial infarction, circulatory shock, brain injury, systemic lupus erythematosus, and hypertension.

6.   A method for controlling vasoconstriction in an animal comprising administering a composition comprising an anti-vasoconstrictive effective amount of LXA$_4$ or an active derivative thereof to said animal.

7.   The method of claim 6, wherein said vasoconstriction is due to activation of a leukotriene receptor.

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8.   The method of claim 7, wherein said receptor is the LTD_4 receptor.

9.   The method of claim 6, wherein said vasoconstriction is the result of release of SRS-A.

10. The method of claim 6, wherein said vasoconstriction is associated with a medical disorder selected from the group consisting of asthma, anaphylactic reactions, allergic reactions, shock, inflammation, rheumatoid arthritis, gout, psoriasis, allergic rhinitis, adult respiratory distress syndrome, Crohn's disease, endotoxin shock, traumatic shock, hemorrhagic shock, bowel ischemic shock, renal glomerular disease, benign prostatic hypertrophy, inflammatory bowel disease, myocardial ischemia, myocardial infarction, circulatory shock, brain injury, systemic lupus erythematosus, and hypertension.

11. The method of claim 5 or 10, wherein said medical disorder is asthma.

12. The method of claim 5 or 10, wherein said medical disorder is an anaphylactic reaction.

13. The method of claim 5 or 10, wherein said medical disorder is an allergic reaction.

14. The method of claim 5 or 10, wherein said medical disorder is renal glomerular disease.

15. The method of claim 5 or 10, wherein said medical disorder is systemic lupus erythematosus.

16. The method of claim 5 or 10, wherein said medical disorder is hypertension.

17. The method of claim 1 or 6, wherein said effective amount is about 0.1 to about 10 mg/kg body weight/day.

18. The method of claim 17, wherein said effective amount is about 0.5 to 5 mg/kg body weight/day.

19. The method of claim 1 or 6, wherein said derivative comprises a covalent substitution at one or more of the three hydroxyl groups of LXA_4.

20. The method of claim 19, wherein said covalent substitution is selected from the group consisting of an acetate, methyl and n-butylboronate substitution.

21. The method of claim 1 or 6, wherein said derivative comprises a covalent substitution at the terminal carboxyl group of LXA_4.

22. The method of claim 21, wherein said covalent substitution comprises esterification of the carboxyl group.

23. The method of claim 1 or 6, wherein said composition is administered parenterally, orally, or topically.

24. The method of claim 23, wherein said parenteral administration is selected from the group consisting of subcutaneous, intravenous, and intraarterial administration.

25. The method of claim 23, wherein said topical administration is selected from the group consisting of rectal and aerosol administration.
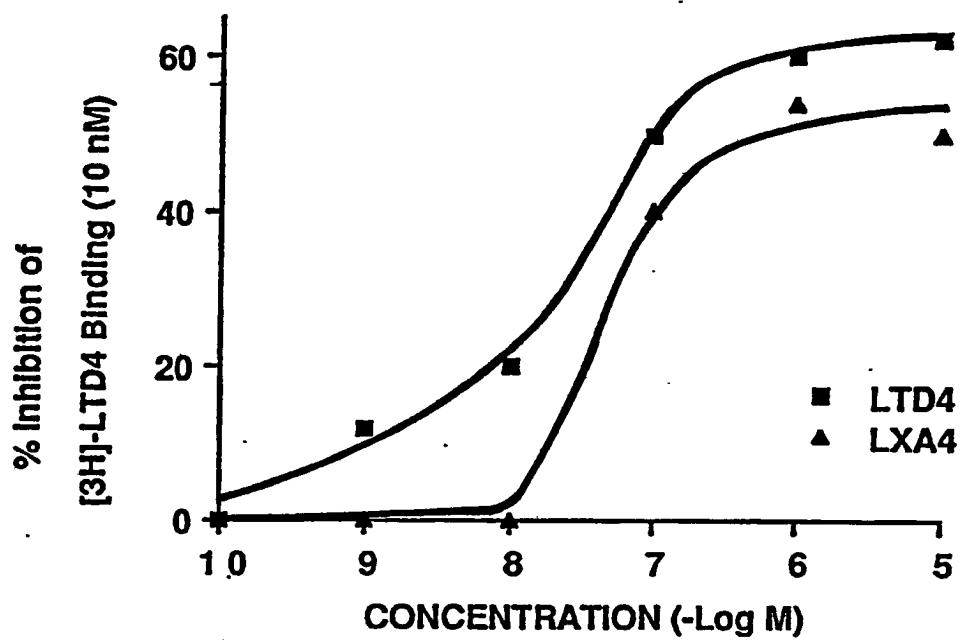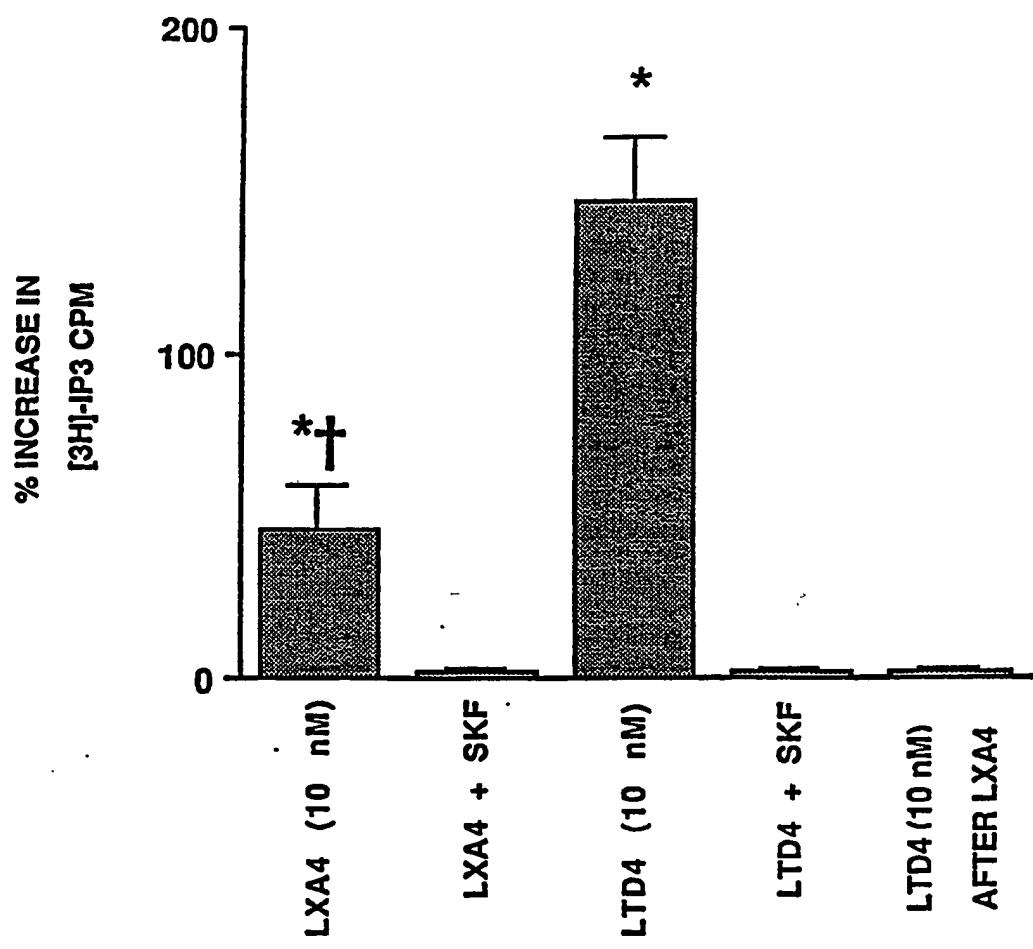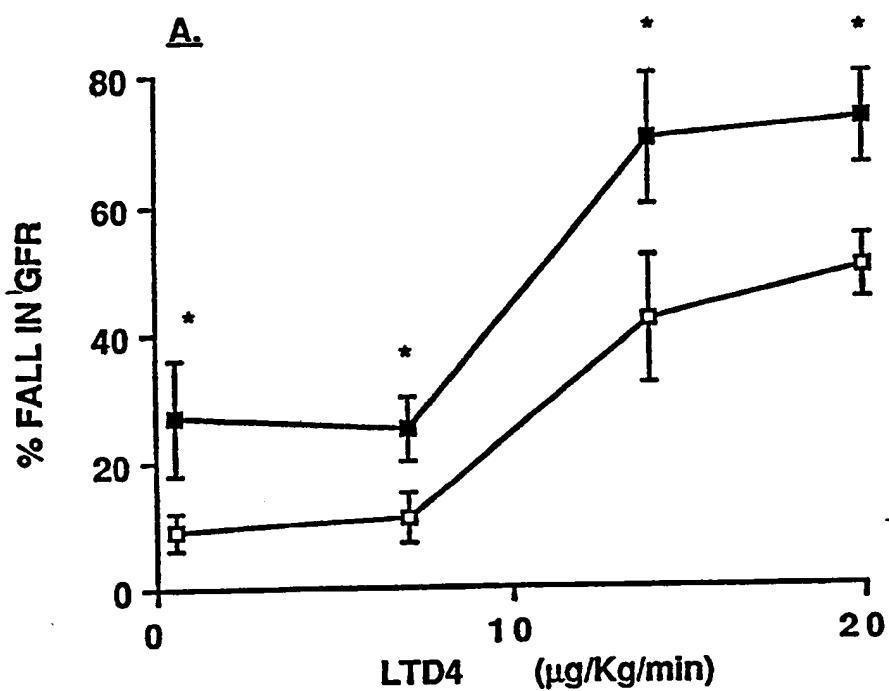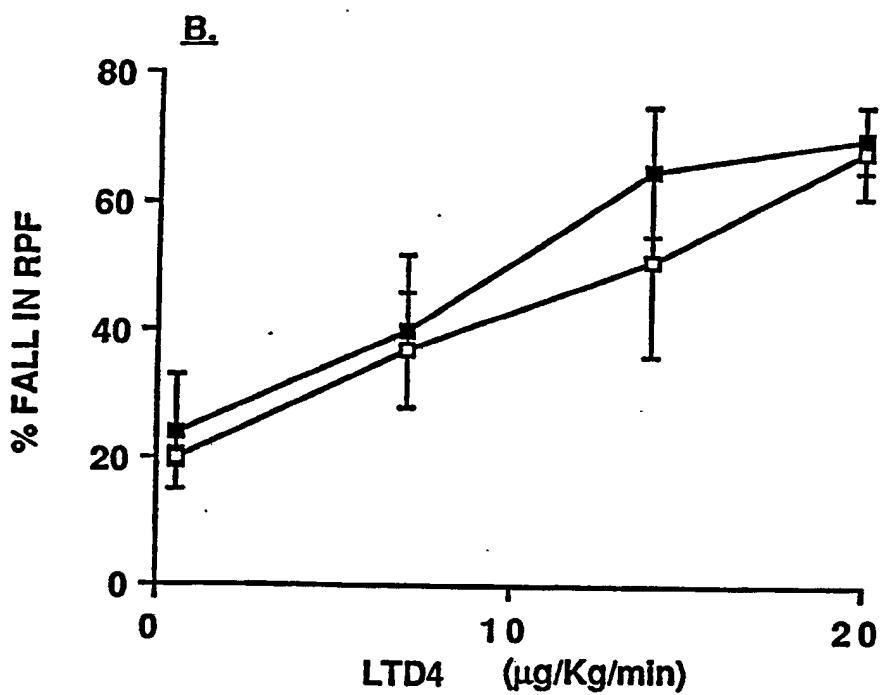
FIGURE 1

FIGURE 2

FIGURE 3A

FIGURE 3B

INTERNATIONAL SEARCH REPORT

International Application No PCT/US90/02320

I. CLASSIFICATION OF SUBJECT MATTER (if several classification symbols apply, indicate all) [3]

According to International Patent Classification (IPC) or to both National Classification and IPC

IPC (5) : A61K 31/23
U.S. Cl : 514/552

II. FIELDS SEARCHED

Minimum Documentation Searched [4]	
Classification System	Classification Symbols
U.S.	514/552

Documentation Searched other than Minimum Documentation
to the Extent that such Documents are Included in the Fields Searched [5]

III. DOCUMENTS CONSIDERED TO BE RELEVANT [14]

Category [*]	Citation of Document, [16] with indication, where appropriate, of the relevant passages [17]	Relevant to Claim No. [18]
A	Chemical Abstracts, Vol. 111, No. 9 (Columbus, Ohio, US,A), (NICOLAU ET AL) "Identification of a novel 7-cis-11-trans-Lipoxin A generated by human neutrophils," see pages 608 and 609, the abstract No. 76329v, Biochim. Biophys. Acta 1989, 1003(1), 44-53 (Eng).	1-25
X	Chemical Abstracts, Vol. 111, No. 1 (Columbus, Ohio, US,A) (BADR ET AL) "Lipoxin A₄ antagonizes cellular and in vivo actions of leukotriene D in rat glomerular mesangial cells: evidence for competition at a common receptor, "see page 529, the abstract No. 5366u, Proc. Natl. Acad. Sci. USA, 1989, 86(9), 3438-42 (Eng).	
X	US,A 4,560,514 (SAMUELSSON ET AL) 24 December 1985 See entire document.	1-25
X	Br. J. Pharmacol. (1988) Vol. 95 see pp. 562-568 Crawford et al.	1-25

* Special categories of cited documents: [15]

"A" document defining the general state of the art which is not considered to be of particular relevance

"E" earlier document but published on or after the international filing date

"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)

"O" document referring to an oral disclosure, use, exhibition or other means

"P" document published prior to the international filing date but later than the priority date claimed

"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention

"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step

"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art.

"&" document member of the same patent family

IV. CERTIFICATION

Date of the Actual Completion of the International Search [2]	Date of Mailing of this International Search Report [3]
24 JULY 1990	2 3 AUG 1990
International Searching Authority [1]	Signature of Authorized Officer [20]
ISA/US	STEPHEN B. MAEBIUS

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